State Wildlife Grant Proposal 2014

State of Illinois

Project Number: T-104-R-1

Project Title: Assessing Wellness in Wildlife Herptile Species in Greatest Need of Conservation

1. Need:

The Illinois landscape has undergone unprecedented change in the last 100 years, and many environments no longer resemble the ecosystems that species evolved in. Declines of several state species have been associated with these landscape changes, however the associated changes in pathogen presence and subsequent ability of habitats to support healthy populations remains largely unknown. Deteriorating wildlife health threatens the sustainability and successfulness of conservation efforts as has been observed in Illinois with White Nose Syndrome (Blehert et al., 2009), Ranavirus (Johnson et al., 2008), and Snake Fungal Disease (Allender et al., 2011). Furthermore, extinction events due to disease, while rare in wildlife, have been documented in both a species of land snail (*Partula turgida*) due to a parasite infestation (Cunningham and Daszak, 1998) and the sharp-snouted day frog (*Taudactylus acutirostris*) due to chytridiomycosis (Schloegel et al., 2006). In both cases, disease outbreaks led to rapid catastrophic declines from which populations could not demographically recover. Neither study described the wellness of individuals in the population prior to the outbreak, which may have allowed a more concerted effort to mitigate disease impact. These disease events may negate the benefits of habitat restoration executed through the IWAP. Thus, conserving the wellness of these populations is integral to conserving ecosystems and assessing recovery efforts and addressing a need identified by the Wildlife Action Team.

The ability to detect changes in the health of an ecosystem requires transdisciplinary cooperation utilizing various approaches, and wildlife sentinels have been proposed as early monitors of ecosystem health (Giulette et al., 1995; Mazet et al., 2000; Sleeman, 2008; Carson et al., 2014; Hamer et al., 2012; Childs et al., 2007). Monitoring the health of sentinel species allows early detection of ecosystem change and directly benefits species health and recovery efforts. Baseline natural history, physiological, and health-related information is needed and is absent from the current WAP.

Techniques that address this data void require a health assessment approach that utilizes specific biomedical diagnostics. Hematologic, plasma biochemical, and pathogen prevalence data have been utilized as a means of determining the wellness of free-ranging reptile populations (Anderson et al., 1997; Wright and Skeba, 1992; Hidalgo-Vila and Ribas, 2011; Brown and Sleeman, 2002; Chaffin et al 2008; Sleeman, 2008; Schrader et al., 2010; Rose and Allender, 2011; Kimble and Williams, 2012), but have not been critically evaluated. In mammals and birds, inflammatory responses observed on

complete blood counts, elevated concentrations of kidney or liver enzymes, and/or presence of pathogens are fairly straightforward (Clarke et al., 2013; Junge and Louis, 2005; Parsons et al., 2005; Turvey et al., 2012; Williams et al., 2011). Unfortunately, assessing health in reptiles is not well-defined and utilizing diagnostic assays designed for mammals often lead to difficulty in interpretation. In addition, the close tie of physiological responses and temperature displayed by ectotherms (Lillywhite, 1987; Peterson et al., 1993) can complicate interpretations compared to endotherms. Baseline studies that establish the same rigor and criteria for interpretation are lacking in Illinois amphibian and reptile populations. The current proposal aims to address these critical needs by collecting baseline hematologic, plasma biochemical, and disease prevalence data that are associated with healthy individuals and populations.

The Illinois WAP identifies species in greatest need of conservation (SGCN), but the wellness of the state's reptiles and amphibians is poorly investigated. To maximize the usefulness of transdiciplinary wellness assessments described above, species response to various environmental conditions need to be investigated simultaneously. Many wildlife studies utilize a single population of individuals at a specific time point. These approaches limit the ability to relate larger ecosystem changes or threats over time or between habitats. The current proposal aims to address this void by evaluating three species in three separate WAP campaigns. Evaluating representative species in these campaigns allows integration of results that may aid in the conservation of other species within the same habitat.

The free-ranging Eastern Box Turtle (*Terrapene carolina carolina*) is listed as vulnerable on the 2011 IUCN Red List, is on Appendix II of CITES and has been proposed as an exemplary sentinel species (CITES Appendix II, 2012; IUCN, 2011; Sleeman, 2008). Eastern Box turtles are distributed across the eastern US in a variety of habitats, have long lifespans, small home ranges, and are slow to reach reproductive maturity, all which may potentiate their susceptibility to environmental stress (Sleeman, 2008) and make them excellent indicator species for environmental change (Schrader et al., 2010; Sleeman, 2008). Moreover, habitat fragmentation, infectious diseases, and toxicological exposure are of increasing concern in box turtles (Brown et al, 2004; De Voe et al., 2004; Johnson et al., 2008; Allender, 2012). Over the past four years, we have been monitoring hematologic, plasma biochemical, contaminant exposure, and pathogen prevalence in Eastern Box Turtles in Illinois. The proposed project would build upon these data and identify ongoing changes that may represent deteriorating environmental conditions or specific conservation threats. Consequently, continuous health monitoring and disease investigations can provide valuable insight to the ecological health as well as aid in the preservation of this species (Brown and Sleeman, 2002; Chaffin et al., 2008; Sleeman, 2008; Schrader et al., 2010).

Currently, there are no epidemiologic studies or systematic multiple pathogen studies on Eastern Box Turtles. This lack of understanding may impact the ongoing conservation strategies for this species and its ecosystem. In a comprehensive epidemiology study investigating ranavirus in box turtles under non-outbreak conditions, prevalence of that pathogen was extremely low (Allender et al., 2013). However, the prevalence of co-

pathogens TeHV1 and *Mycoplasma* has been shown to be high in these populations (Ossiboff et al., 2015). These pathogens are less likely to cause mortality and may better represent individual or environmental stress. Disease ecology of each individual pathogen is largely unknown, and management strategies that target mitigation of a single disease, without considering the interaction of other pathogens may be grossly over simplified. The proposed research will fill a critical need in describing the pathogen prevalence of several known and unknown Eastern Box Turtle pathogens that will aid in the overall conservation strategies of this species.

Evaluating the health of the box turtle can enhance other sympatric species utilizing different resources within the same environment, as has been demonstrated for other chelonian (Sibernagel et al., 2013) and wildlife (Herrera et al., 2008; Junge and Louis, 2005; Navarro-Gonzalez et al., 2014). The Silvery Salamander (*Ambystoma platineum*) co-occurs with the Eastern Box Turtle within the Vermillion County Conservation Opportunity Area (COA). The Silvery Salamander is also an excellent indicator of environmental change. It has a bi-phasic life cycle and as such lives in both aquatic and terrestrial habitats, transferring energy and contaminants between habitats. In addition, its permeable skin makes it sensitive to all environmental contamination. Disease threats may be similar for both species, but the response, role in disease ecology, and threat to sustainability differ. Other threats to the wellness of the Silvery Salamander include *Batrachochytrium dendrobatidis* (*Bd,* chytrid), a global amphibian disease agent (Skerratt et al., 2007) that has been identified in the Vermillion COA, although not in the Silvery Salamander (Beyer et al., 2015). Recently in Europe, a larger threat to salamander conservation was discovered, *Batrachochytrium salamandrivorans,* but has yet to be detected in the US, (Martel et al., 2013). However, the existence of these pathogens in the environment is a conservation threat and the baseline health of this species prior to disease emergence might allow efforts that mitigate the efforts if introduced.

While several studies exist on the health or disease occurrence in the Eastern Box Turtles, fewer exist on the Ornate Box turtle (*Terrapene ornata*), despite significant declines across its range (Cureton et al., 2014). Historical occurrences of mortality events (Metcalf and Metcalf, 1979), disease events (Christiansen et al., 2004; Farkas and Gal, 2009), and physiological responses (heart rate, respiratory rate) to temperature and other demographic factors (Bachman, 2013; Bethea, 1971) have occurred in this species. Furthermore, a current disease threat has been minimally investigated within Illinois (Nachusa; Allender unpub data). Despite these reports, there is a paucity of information on other disease threats or wellness in this species anywhere across the range, including Illinois. Utilizing similar techniques applied to the Eastern Box Turtle will enable integration of the data sets in species with similar physiology and possibly elucidate different approaches to maintaining wellness.

There are several threats to the success of conservation programs for the Eastern Box Turtle, Ornate Box Turtle, and Silvery Salamander. Each of these species has current or historical observations of poor health or disease susceptibility. Furthermore, the habitats of each of these species have been identified by the WAP as locations with significant existing, or potential wildlife habitat resources. Utilizing three species that overlap

habitat, natural history, and disease threats allows integration of health results that could potentially contribute to the success of their conservation.

2. Purpose and Objectives:

The purpose of this project is to assess the health of the Eastern Box Turtle, Ornate Box Turtle, and Silvery Salamander through the generation of baseline hematology and disease prevalence data. This health monitoring will establish criteria that can be integrated into future conservation assessments of SGCN. Our specific objectives are:

1. Identify three herptile SGNC in three separate campaigns (Forests and Woodlands; Prairie and Farmland; Wetlands) in consultation with campaign leads that fit the criteria of sample size, number of populations, and current natural history data.

2. Establish baseline health profiles for the SGNC identified in Job 1
    a. Utilize hematology, plasma biochemistry, and protein electrophoresis to characterize the general health of in three campaigns.
    b. Establish baseline prevalence of common pathogens
    c. Provide technical resources training for IDNR staff to characterize baseline health and its impacts

3. Assess the occurrence of emerging or ongoing infectious diseases in SGNC
    a. Surveillance of pathogens in SGNC populations in at least three campaigns
    b. Investigate mortality events in SGNC as they occur
    c. Provide technical resource training for IDNR staff and partners through webinars, staff presentations, or onsite training in disease detection and response

3. Expected Results or Benefits:

This project will provide data necessary for assessing the success of the IWAP. First, this effort will locate at least one population of each of three SGNC for development of a health assessment protocol. Each health assessment will produce reference ranges of white blood cell types, the normal PCV (low values indicate anemia), and one metabolic/inflammatory protein value (total protein) in the blood. In addition, each species (and population where applicable) will have data produced for six biochemical analytes that evaluate liver (two analytes), kidney, and blood calcium and phosphorus.

For each species, Ranavirus prevalence will be determined. In addition Terrapene herpesvirus 1 and Mycoplasma prevalence will be determined in both box turtle species, and *Batrachochytrium dendrobatidis* and *B. salamandrivorans* prevalence will be determined in the Silvery Salamander.

This project will produce a protocol for assessing wellness in representative reptile and amphibian SGCN targeted by each campaign that can serve as guide for other taxa in each campaign. This research will produce three presentations that will be given by WEL within the region of the project and one in Springfield for land managers, veterinarians, and IDNR that identify and prevent diseases before they threaten wildlife and affect humans. The information gathered will provide a point of reference from which to measure the future impacts of these diseases should they intensify or spread. It will identify conservation actions/best practices that could be incorporated in to the Action Plan and be used when staff are reintroducing animals or restoring habitat to minimize disease risk and enhance health.

4. Approach:

Species and Population Sampling – State agency (IDNR) staff, IWAP campaign leads, and WEL will identify at least three species of herptiles in three IWAP campaigns (Wetlands, Farmland & Prairie, Forests & Woodlands) for investigation. An attempt to identify several populations of each species will be made. If multiple populations of a species exist, but habitat improvement is in a different stage among the populations, they will be sampled equally to determine baseline wellness under differing conditions. Preliminary discussions with the Campaign leads have identified the Eastern Box Turtle (*Terrapene carolina carolina*) in the Vermillion COA and Stephen A. Forbes State Park, the Ornate Box Turtle (*Terrapene ornata*) in the TNC Nachusa Grasslands Preserve, and the Silvery Salamander (*Ambystoma platinuem*) in the Vermillion COA.

Health and Hematologic Assessments – Each animal will be assigned a permanent ID and mass, sex, and age status recorded. Straight carapace length (SCL), straight carapace height (SCH), and carapace width (CW) will be determined in the box turtles, while mass and snout-vent length (SVL) will be recorded for the salamanders. Physical examinations will be performed, noting visual appearance of the eyes, nose, oral cavity, legs, digits, shell, and integument. A whole blood sample will be taken from the subcarapacial sinus of the box turtles and the caudal tail vein in the Silvery Salamander. Blood will be placed in lithium heparin microtainers, and transported on wet ice until analysis later in the same day.

Packed cell volume (PCV) and total solids (TS) analysis will be performed by filling two sodium heparinized microhematocrit tubes (Jorgensen Laboratories,Inc., Loveland, CO 80538) from one LH microtainer tube. Each sample will be centrifuged (14,500 rpm x 5 minutes) and the percent red blood cell (RBC) recorded. Total solids will be determined by refractometer (Amscope RHC-200ATC refractometer, National Industry Supply, Torrance, CA, USA) using plasma in the microhematocrit tube. Total white blood cell (WBC) counts will be determined using an Avian Leukopet (Vet lab Supply, Palmetto Bay, FL, USA) on a Bright-line hemacytometers (Hausser Scientific, Horsham, PA, USA) following the manufacturer's protocol. Fresh blood smear slides will be stained with a modified Wright's Geimsa stain and one hundred white blood cell differential counts will be performed by a single observer.

Plasma biochemical analysis will be performed using a Beckman Coulter AU680 at University of Illinois Clinical Pathology Laboratory. Analysis includes the variables

calcium, phosphorus, aspartate aminotransferase, bile acids, creatine kinase, and uric acid.

Protein electrophoretic profiles will be performed on plasma submitted to the University of Miami or University of Illinois clinical pathology laboratory. Briefly, plasma samples will be analyzed according to the procedure provided by the Helena SPIFE 3000 system with the use of Split Beta gels (Helena Laboratories, Inc., Beaumont, TX, USA). Results will produce after gel scanning and analysis by Helena software. Fraction delimits will be placed as previously demonstrated for other reptiles (Gimenez et al., 2010). Percentages for each fraction will be determined by this software and absolute values (g/dl) for each fraction will be obtained by multiplying the percentage by the total protein concentration. The A/G ratio will be calculated by dividing albumin by the sum of the globulin fractions. Total protein will be determined by a non-temperature compensated refractometer (Shuco, Japan).

Disease Detection – Whole blood and oral swab samples in all three species will be assayed for ranavirus using qPCR, while oral swabs from the box turtles will be assayed for Terrapene herpesvirus 1 (TeHV1), and Mycoplasma
(Allender et al., 2013; Ossiboff et al., 2015). In salamanders only, *Batrachochytrium denrobatidis* (Skerratt et al., 2007) and *Batrachochytrium salamandrivorans* (Martel et al., 2013) have emerged as a serious threat to amphibian populations and will be assayed through qPCR of skin swabs.

Geospatial Analysis: All mapping and analysis will be performed using ArcGIS software (ArcGIS 10.1, ESRI, Redlands, CA 92373). Basemaps will be acquired from the Illinois Spatial Data Server. County boundary shapefiles will be added to the map, along with polygon layers of Natural Resource lands, geology, streams, and landcover. Ortho images will be used to demonstrate urban features more accurately. Original data points (that corresponded to capture location of each turtle) will be entered into an excel spreadsheet in degree minutes and imported into ArcCatalog. Known locations of pathogens will be mapped using satellite images and point data. A data set of weighted features will then be created based on variables such as packed cell volume, total dissolved solids, and white blood cell count. The hot spot analysis tool identifies statistically significant hot spots and cold spots using Getis-Ord Gi* statistic, which will then be performed for each pathogen. It creates a new output feature class with a z-value and p-value for each. Z-values are an illustration of the standard deviations and p-value is the probability the observed pattern was created by a random process. This analysis will graphically illustrate prevalence of the disease and health parameters that could be influencing infection across a region.

Mortality Events: IDNR and WEL will report mortality events in herptiles throughout the state. WEL will collect specimens and environmental variables. Necropsy will be performed on suitable carcasses and samples collected for diagnosis. Follow-up investigation by WEL or IDNR staff of remaining individuals in the population will depend on initial findings.

Technical Resource Training: Annual hour-long presentations will be delivered in consultation with DNR officials within the region of the project or in Springfield. These presentations will focus on keeping DNR officials up to date on information for real-time responses to threats, but also evolve into discussions about future efforts and needs.

5. Useful Life:

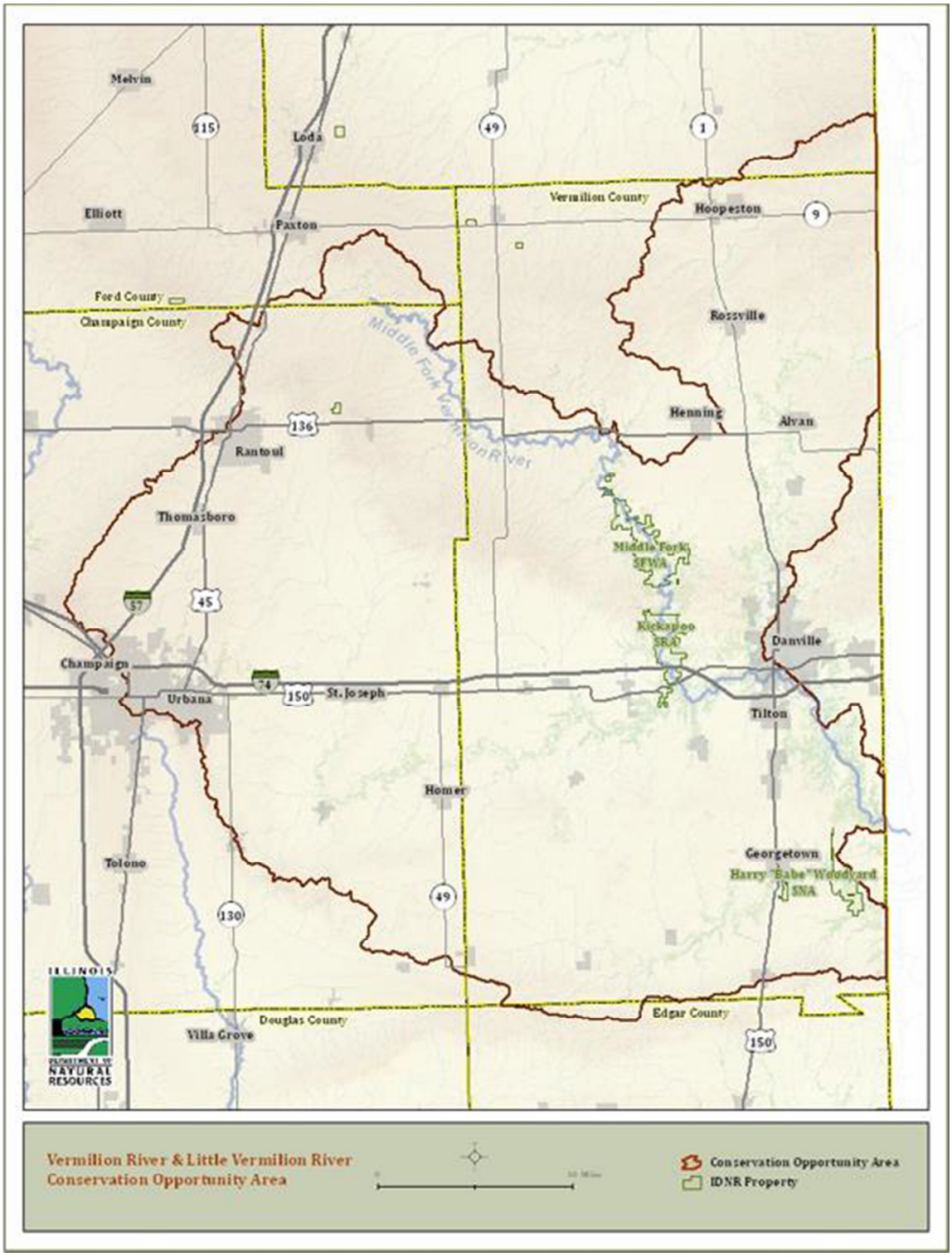
This project is not a capital improvement project of greater than $100,000 so this section does not apply.

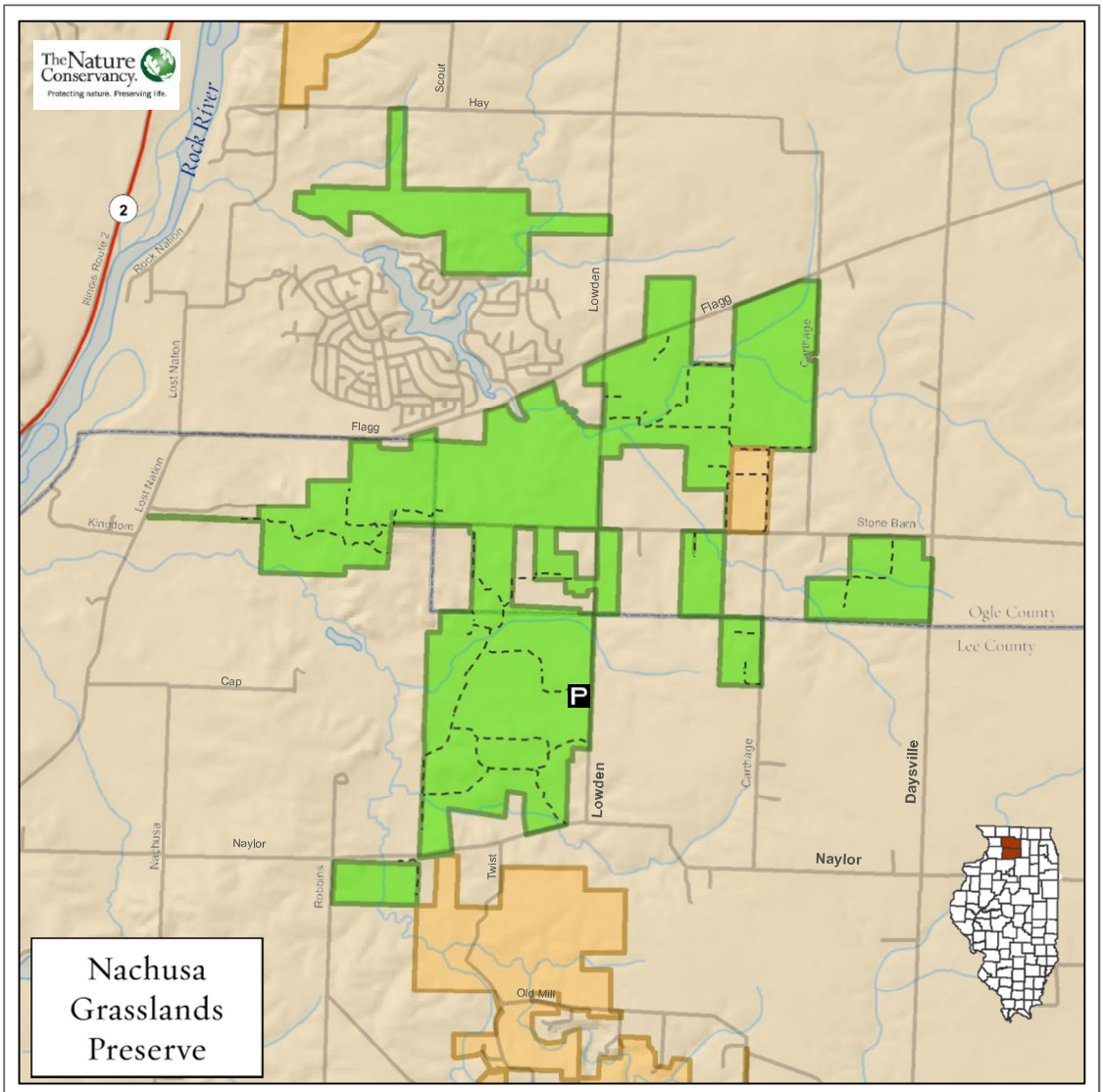
6. Geographic Location:

The Vermilion River Conservation Opportunity Area (VR COA) covers 791,665 acres (1,231 square miles) in Champaign, Vermilion, Ford, Edgar, and Iroquois Counties. The VR COA lies within both the Grand Prairie Natural Division and the Vermilion River section of the Wabash Border Natural Division described in the IWAP. The majority of the land is privately owned and monoculture agricultural production dominates the landscape. The Vermilion River (Salt Fork, Middle Fork, and North Fork) as well as the Little Vermilion River lie within the boundaries of the VR COA. The Middle Fork is the only designated National Wild and Scenic River in Illinois. Additionally, parts of the VRCOA area include portions of Indiana in Warren, Benton, and Vermillion counties.

Nachusa Grasslands consists of 3,100 acres of prairie remnants, restorations, and reconstructions located between Oregon, Dixon and Franklin Grove, IL. Remnant prairie knobs were protected from the plow by an unfarmable overlay of St. Peter Sandstone. Starting in 1986 with the purchase of 250 acres, The Nature Conservancy has gradually recreated a vision of 1800 Illinois' mosaic of prairie, savanna and wetlands. Hundreds of dedicated volunteers have collected seed to replant former corn and bean fields.

The Stephen A. Forbes State Recreation Area, located 15 miles northeast of Salem in Marion County, consists of a total park area to 3,103 acres. Of this total, 1,150 acres are forests of oak and hickory which surround a large lake. The lake was completed in 1963 and has 18 miles of shoreline.


Melvin
115
Loda
49
1
Vermilion County
Hoopeston
9
Elliott
Paxton
Ford County
Champaign County
Middle Fork Vermilion River
Rossville
Henning
Alvan
136
Rantoul
Thomasboro
Middle Fork SFWA
57
45
Kickapoo SRA
Danville
Champaign
74
150
St. Joseph
Urbana
Tilton
Homer
Georgetown
Harry "Babe" Woodyard SNA
Tolono
49
130
ILLINOIS
DEPARTMENT OF NATURAL RESOURCES
Douglas County
Edgar County
Villa Grove
150
Vermilion River & Little Vermilion River
Conservation Opportunity Area
Conservation Opportunity Area
IDNR Property



Preserve Boundary
IL DNR Owned
Hiking Trails
Roads
Streams
Lakes & Rivers
Parking

N

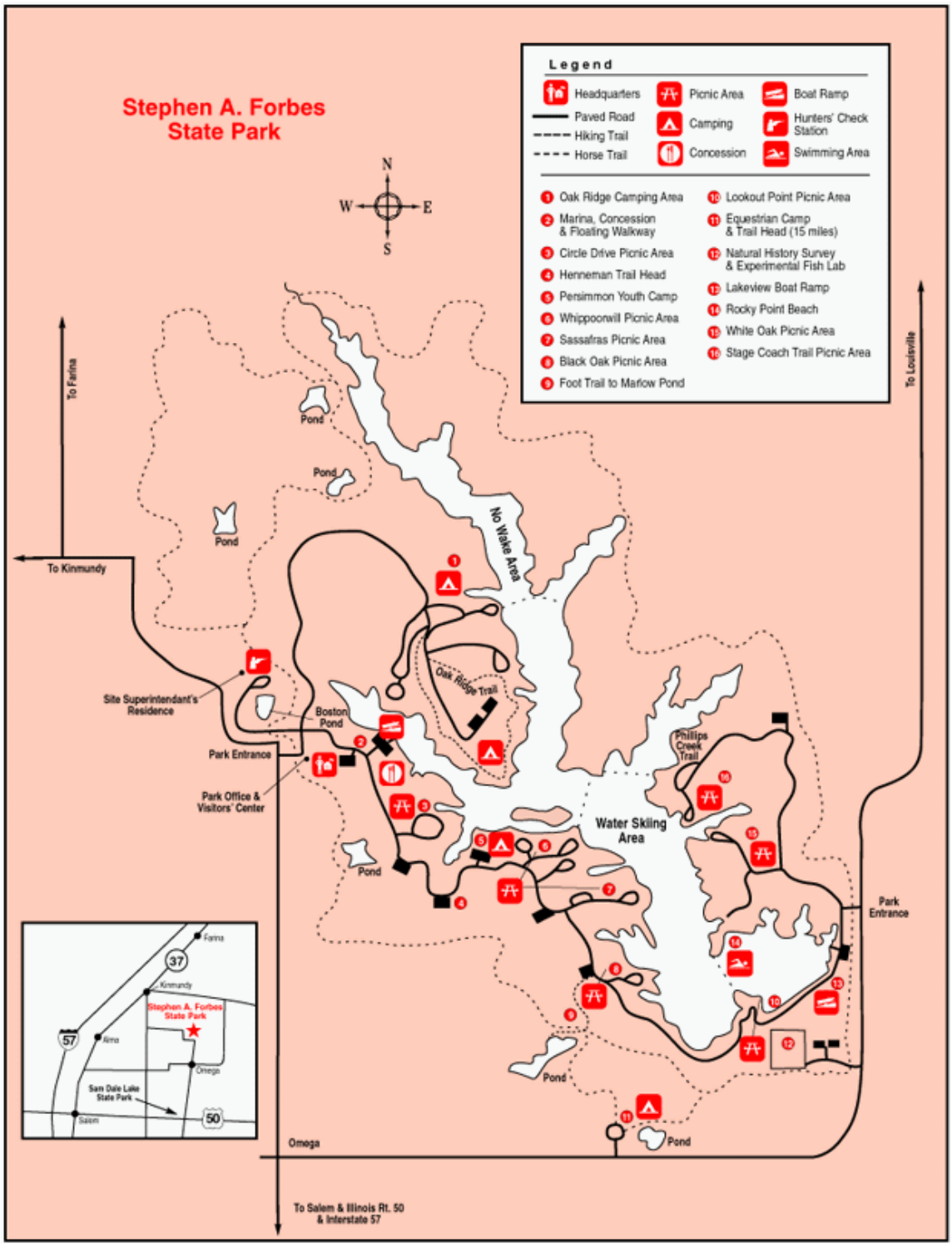
0 0.5 1 Miles

From the East: I-88 West; Exit at Route 251 North (Rochelle); take Route 38 West; travel into Franklin Grove, go two blocks past Casey's gas station and turn right (north) at Daysville Road/1700E (sign: Franklin Creek State Park); travel 1.5 miles north to Naylor Road/1950 North, then turn left (west) and go 2.2 miles to Lowden Road/1500 East; turn right (north) and go one mile to entrance on left

From the South: I-39 North; take Route 38 West; then, follow previous directions.

From the North: I-39 South; Route 64 west toward Oregon; LEFT Daysville Road/1700 East; RIGHT onto Lowden Road/1500 East, take to entrance on the west (right) side of the road.

September 2011


Stephen A. Forbes
State Park
Legend
Headquarters
Picnic Area
Boat Ramp
Paved Road
Camping
Hunters' Check Station
Hiking Trail
Horse Trail
Concession
Swimming Area
1 Oak Ridge Camping Area
2 Marina, Concession & Floating Walkway
3 Circle Drive Picnic Area
4 Henneman Trail Head
5 Persimmon Youth Camp
6 Whippoorwill Picnic Area
7 Sassafras Picnic Area
8 Black Oak Picnic Area
9 Foot Trail to Marlow Pond
10 Lookout Point Picnic Area
11 Equestrian Camp & Trail Head (15 miles)
12 Natural History Survey & Experimental Fish Lab
13 Lakeview Boat Ramp
14 Rocky Point Beach
15 White Oak Picnic Area
16 Stage Coach Trail Picnic Area
N
W
E
S
To Farina
To Louisville
To Kinmundy
Pond
Pond
Pond
No Wake Area
Oak Ridge Trail
Site Superintendant's Residence
Boston Pond
Park Entrance
Park Office & Visitors' Center
Phillips Creek Trail
Water Skiing Area
Pond
Park Entrance
Pond
Pond
Omega
To Salem & Illinois Rt. 50 & Interstate 57
Farina
37
Kinmundy
Stephen A. Forbes State Park
57
Xenia
Omega
San Dale Lake State Park
Salem
50

7. Principle Investigator:

Dr. Matt Allender, DVM, PhD, Clinical Assistant Professor, Zoo and Wildlife Veterinarian, University of Illinois, College of Veterinary Medicine, 2001 S. Lincoln Avenue, Urbana, IL 61802. Phone: 217-265-0320. Email: mcallend@illinois.edu.

Dr. Christopher Phillips, PhD, Research Program Leader, University of Illinois, Prairie Research Institute, Illinois Natural History Survey, 1816 S. Oak St., Champaign, IL 61820.

8. Program Income:

This grant will generate no additional income therefore this section does not apply.

PROJECT BUDGET

Project Title: Assessing Wellness in Wildlife Herptile Species in Greatest Need of Conservation
Project Number: T-104-R-1
Project Time Frame: Start Date - (10\01\2015); End Date - (09\30\2018)

Budget Categories		Federal Funds	Non-Federal Funds	Totals
Salaries and Wages		$22,467.00	$11,879.00	$34,7346.00
Fringe Benefits (Fellow private insurance reimbursement)		$18,033.00	$5,318.00	$23,351.00
Travel		$12,000.00	$0.00	$12,000.00
Equipment		$0.00	$0.00	$0.00
Materials and Supplies		$72,063.00	$0.00	$72,063.00
Contractual Services		$22,500.00	$0.00	$22,500.00
Other:	Federal: Fellow Stipend	$108,182.00		$177,419.00
	Non- Fed = Foregone Tuition Remission @ 64%		$69,237.00	
Total Direct Costs		$255,245.00	$86,434.00	$341,679.00
Modified Total Direct Cost (MTDC)		$147,063.00	$17,197.00	$164,260.00
Indirect Rate of 20%		$29,413.00		$29,413.00
Indirect Rate of 58 6_ %			$10,077.00	$10,077.00
Unrecovered Indirect Rate (20% vs 58 .6_ % MTDC)			$56,766.00	$56,766.00
Total Project Costs		$284,658.00	$153,277.00	$437,935.00
Percentage of Total Project Cost		65.00%	35.00%	100.00%

BUDGET JUSTIFICATION

Salaries and Wages:

This project would support a fellowship for a graduate veterinarian to pursue a PhD through the Wildlife Epidemiology Lab at the University of Illinois (WEL). The fellow will work with the PI and campaign leads to identify species in Objective 1. He/She will also calculate sample size and determine populations to sample in Objective 1. The fellow will perform fieldwork, and collect blood, swab, and/or tissue samples for Objective 2 on all populations established in Objective 1. The fellow will be the contact person for emerging and ongoing outbreaks in Objective 3. The fellow will perform the investigation and diagnostic work-up for outbreaks in Objective 3. The fellow will develop a series of training presentations surrounding the need and execution for wildlife health monitoring and reporting. The fellow will take and teach modeling, wildlife health, and natural resource courses that help to identify emerging and ongoing disease events and that help to enable analysis of data generated in Objectives 2 and 3. Similarly, the fellow will report these findings at national conferences that will enhance the success of Objectives 2 and 3 by interacting with colleagues in the field that may have information that can be incorporated into executing Objective 2 and 3. The fellow will be responsible for finding funding for ongoing outbreaks identified in Objective 3 or exploring possible diagnostic modalities that can be incorporated in to Objective 2. These are all consistent with the opportunities offered through additional training.

Student hourlies will be hired to assist with field work. They will be paid $12/hr and their hours will not exceed the $7500 per year budgeted. $113,133.33/yr for 3 years. = $3959.67/yr x 3 yrs = $11,879.00 (rounded)

3.5% of the PI's time (Salary and Benefits) @ $113,133.33 will be contributed as match to this project

Fringes:

Fellows are not considered employees in Illinois. Therefore there are no fringe rates to apply to the fellow's stipend. However, we have budgeted to reimburse the cost of private health coverage for the fellow. $500 per month is estimated to cover this for a total of $18k. Fellow's reimbursement will be less the standard amount each employee contributes toward premiums each month.

PI's benefits are University of Illinois full rate of 44.77%. The PI benefits are a Non-Federal match.

Student hourlies are only assessed .15% (Worker's Comp) for fringes @ $33.00.

Equipment:

N/A

Materials and Supplies:

Objective 1: The project will require field supplies, such as weatherproof paper, batteries, PIT tags (or other identifiers), and other such consumables needed to conduct the project's field aspect. (~ $6,000 per year) x 3 years = $18k

Objective 2: Whole blood and swab collection and processing supplies for an expected 200 samples each year that will be collected at all sites combined include polypropylene tubes, storage boxes, cotton-tipped applicators, syringes (3 ml), needles (22 ga), vacutainers (lithium heparin), and slides. (600 x $10/ea = $6,000)

Health assessment is budgeted for complete blood counts for up to 640 samples. Plasma biochemistries are analyzed in the Clinical Pathology lab at the College at $14/sample for 640 samples. (640 x $14 = $8,960)

Quantitative PCR extraction is budgeted for 640 samples (320 each of blood and swabs) using Qiagen Extraction kit (DNA mini blood kit). (640 x $2.75 = $1,760)

TaqMan primer-probe sets are budgeted at $255/set for a maximum of 32 sets (16 pathogens in duplicate). (32 x $255 = $8,160)

Reaction costs are run in triplicate using TaqMan qPCR Supermix with reagents on either the Fluidigm real-time thermacylcer ($675 per every 40 samples) at the Keck Biotechnology Center or ABI real-time thermacycler maintained in the College of Veterinary Medicine at the University of Illinois. (640/40 = 16 x $675 = $10,800)

Gel electrophoresis to identify the specific disease is budgeted at $100/sample for up to 20 samples. (20 x $100 = $2,000)

Plasticware includes 96 well optical reaction plates and MicroAmp Optical adhesive film. Miscellaneous supplies include pipette filter tips, gloves, and eppendorf tubes ($5/sample).
(640 x $5 = $3,200)

Objective 3: The project will need supplies for tissue collection, histopathology, and disease investigation. Necropsy including microbial culture and isolation is performed in the diagnostic lab within the College of Veterinary Medicine and budgeted at $250/animal for up to 50 animals.
(50 x $250 = $12,500)

The project will also require general office supplies such as pens, pencils, and printer cartridges necessary to complete the project. (~$600)

Contractual Services:

Contractual services for this project include veterinary tests conducted through outside laboratories (Objective 3 - plasma electrophoresis 50 x $56 = $2,800) and canine search team for box turtles (Objective 2 - $1600/week x 4 wks/yr x 3 years = $19,200), shipping charges of the samples for the health studies, computer software, printing and duplicating costs associated with report writing and publication of results in the peer-reviewed literature ($500 for page charges, reprints).

Travel:

Travel expenses for this project will be for travel to and from project field sites (within the state of Illinois). Expenses will include mileage expenses while using state, leased, or personal vehicles at the University established rates. Housing at the field sites (if necessary) is budgeted at state of Illinois nightly rates for an estimated 10 nights per year.

Facilities and Administration (F & A)

20% is assessed on the total direct costs of the Federal request. Unrecovered F&A is calculated as the difference between the current full on-campus research rate of 58.6%, less the negotiated rate, plus 58.6% on the MTDC of the match items.

Other:

Fellowship stipend = $108,182.00. Forgone tuition remission (64% surcharge added to fellowship stipend - $69,236)

Tuition remission of 64% of Fellow's stipend would normally be charged to the award as partial reimbursement to the University. That foregone tuition remission is noted as part of the match. (Fellow Stipend = $35,000 Yr 1 + $36,050 Yr 2 + $37,132 Yr 3 = $108,182 . Forgone Tuition Remission as match = $108,182 x 64% = $69,236.00.)

10. Multipurpose Project:

This project does not qualify as a multipurpose project therefore this section is not applicable.

11. Relationship with Other Grants:

There is no relationship with this grant for any other project underway, planned, or anticipated funded by Federal grants. There is overlap with the state Wildlife Preservation Fund project (15-LW15) that is investigating the role of ranavirus and herpesvirus co-infections in Eastern Box Turtles in Vermillion COA.

12. Timeline:

October 2015 – January 2016	Confirm species of focus with Campaign Leads
February 2016 – October 2016	Perform field work
November 2016	Compile data and present to DNR
February 2017 – October 2017	Perform field work
November 2017	Compile data and present to DNR
February 2018 – October 2018	Perform field work
November 2018	Compile data and present to DNR
December 2018	Prepare final report

13. General:

(i) **Substantial in Character and Design**

The project statement describes a need consistent with the -State Wildlife Grants (SWG); states a purpose and sets objectives, both of which are based on the need; uses a planned approach, appropriate procedures and research; and is cost effective.

(ii) **Compliance:**

The IDNR will use its CERP (Comprehensive Environmental Review Process) as a tool to aid the Department in meeting NEPA compliance for the project outlined under this grant proposal. It is the Department's policy to require CERP applications for all land disturbing activities unless those activities are covered by CERP exemptions.

All planned activities will also be in compliance with the Endangered Species Act. All determinations and documentation will be in accordance with the current established U.S. Fish and Wildlife Service protocols for section 7.

All planned activities will be in compliance with the National Historic Preservation Act and the Council on Historic Preservation Act. All determinations and documentation will be in accordance with the terms of the Programmatic Agreement, as amended, effective September 23, 2002.

When applicable, those planned activities which involve a floodplain and/or jurisdiction wetlands will be done in accordance with Presidential Executive Orders 11988 and 11990.

When applicable, those planned activities which involve programs and/or site improvements will be done in accordance with Section 504 of the Rehabilitation Act and the Americans with Disabilities Act.

When applicable, those planned activities which involve the use of pesticides, herbicides or other comparable chemicals will be done in accordance with current state and federal regulations to assure the safe and legal application of those chemicals. All chemicals will be applied in accordance with the manufacturers label instructions. All persons applying chemicals will be licensed by the Illinois Department of Agriculture as a chemical operator along with a licensed applicator, in accordance with Illinois state law.

(iii) References

Allender MC. 2012. Characterizing the Epidemiology of Ranavirus in North American Chelonians: Diagnosis, Surveillance, Pathogenesis, and Therapy. PhD Dissertation, University of Illinois.

Allender MC, Dreslik M, Wylie S, Phillips C, Wylie D, Maddox C, Delaney MA, Kinsel MJ. 2011. An unusual mortality event associated with *Chrysosporium* in Eastern Massasauga Rattlesnakes (*Sistrurus catenatus catenatus*). *Emerg Infect Dis* 17:2383—2384.

Allender MC, Mitchel MA, McRuer D, Christian S, Byrd J. 2013. Prevalence, clinical signs, and natural history characteristics of frog virus 3-like infections in eastern box turtles (*Terrapene carolina carolina*). Herp Cons Biol 8: 308-320.

Anderson NL, Wack RF, and Hatcher R. 1997. Hematology and clinical chemistry reference ranges for clinically normal, captive New Guinea snapping turtle (*Elseya novaeguineae*) and the effects of temperature, sex, and sample type. Journal of Zoo and Wildlife Medicine 28: 394–403.

Bachman, G. 2013. Cooling and hiberation effects on leukocyte numbers in the Ornate box turtle *Terrapene ornata ornata*. SICB Annual Meeting Abstracts P1.187, San Francisco, CA.

Bethea, NJ. 1971. Effects of temperature on heart rate and rates of cooling and warming in *Terrapene ornata*. Comp Biochem Physiol 41A: 301-305.

Beyer, S.E., C.A. Phillips, and R.E. Schooley. Canopy cover and drought influence the landscape epidemiology of an amphibian chytrid fungus. Ecosphere, in press.

Blehert DS, Hicks AC, Behr M, Meteyer CU, Berlowski-Zier BM, Buckles EL, Coleman JTH, Darling SR, Gargas A, Niver R, Okoniewski JC, Rudd RJ, Stone WB. 2009. Bat white-nose syndrome: an emerging fungal pathogen? *Science* 323:227.

Brown JD, Sleeman JM. 2002. Morbidity and mortality of reptiles admitted to the wildlife center of Virginia, 1991 to 2000. Journal of Wildlife Diseases 38: 699–705.

Brown JD, Richards JM, Robertson R, Holladay S, and. Sleeman JM. (2004). Pathology of aural abscesses in free living eastern box turtles (*Terrapene carolina carolina*). Journal of Wildlife Diseases 40: 704–712.

Carson C, Lavender CJ, Handasyde KA, O'Brien CR, Hewitt N, Johnson PDR, and Frye JAM. 2014. Potential wildlife sentinels for monitoring the endemic spread of human Burui ulcer in South-East Australia. PLOS Neglected Tropical Diseases. DOI: 10.1371/journal.pntd.0002668.

Chaffin K, Norton TM, Gilardi K, Poppenga R, Jensen JB, Moler P, Cray C, Dierenfeld ES, Chen T, Oliva M, Origgi FC, Gibbs S, Mazzaro L, Mazet J. 2008. Health assessment of free-ranging alligator snapping turtles (*Macrochelys temminckii*) in Georgia and Florida. Journal of Wildlife Diseases 44: 670–686.

Childs JE, Krebs JW, Real LA, and Gordon ER. 2007. Animal-based national surveillance for zoonotic disease: quality, limitations, and implications of a model system for monitoring rabies. Preventative Veterinary Medicine 78: 246–261.

Christiansen JL, Grzybowski JM, Rinner BP. 2004. Facial lesions in turtles, observations on prevalence, reoccurrence, and multiple origins. J Herp 37: 293-298.

Clarke J, Warren K, Calver M, de Tores P, Mills J, Robertson I. 2013. Hematologic and serum biochemical reference ranges and an assessment of exposure to infectious diseases prior to translocation of the threatened western ringtail possum (*Pseudocheirus occidentalis*). J Wildl Dis. 49(4):831-40.

Cunningham AA, Daszak P. 1998. Extinction of a species of land snail due to infection with a microsporidian parasite. *Conserv Biol* 12:1139—1141.

Curreton JC, Janis M, Lutterschmidt WI, Randle CP, Ruthven DC, Deaton R. 2014. Effects of urbanization on genetic diversity, gene flow, and population structure in the ornate box turtle (*Terrapene ornata*). Amphibia-Reptilia 35: 87-97.

De Voe R, Geissler K, Elmore S, Rotstein D, Lewbart G, Guy J. 2004. Ranavirus-associated morbidity and mortality in a group of captive eastern box turtles (*Terrapene carolina carolina*). Journal of Zoo and Wildlife Medicine 35: 534–543.

Farkas SL, Gal J. 2009. Adenovirus and mycoplasma infection in an ornate box turtle (*Terrapene ornata ornata*) in Hungary. Vet Micro 138: 169-173.

Gimenez, M, Saco Y, Pato R, Busquets A, Martorell JM, and A. Bassols. 2010. Plasma protein electrophoresis of *Trachemys scripta* and *Iguana iguana*. Vet. Clin. Path. 39: 227-235.

Guilette LJ, Crain DA, Rooney AA, and Pickford DB. 1995. Organization versus activation: the role of endocrine-disrupting contaminants (EDCs) during embryonic development in wildlife. Environmental Health Perspectives 103, S7: 157-164.

Hamer SA, Lehrer E, and Magle SB. 2012. Wild birds as sentinels for multiple zoonotic pathogens along an urban to rural gradient in greater Chicago, Illinois. Zoonoses Public Health 59: 355–364.

Herrera HM, Abreu UG, Keuroghlian A, Freitas TP, Jansen AM. 2008. The role played by sympatric collared peccary (*Tayassu tajacu*), white-lipped peccary (*Tayassu pecari*), and feral pig (*Sus scrofa*) as maintenance hosts for *Trypanosoma evansi* and *Trypanosoma cruzi* in a sylvatic area of Brazil. Parasitol Res. 103(3):619-24.

Hidalgo-Vila J, Martínez-Silvestre A, and Ribas A. 2011. Pancreatitis associated with the helminth Serpinema microcephalus (Nematoda: *Camallanidae*) in exotic Red-Eared slider turtles (*Trachemys scripta elegans*). Journal of Wildlife Diseases 47: 201–205.

Johnson AJ, Pessier AP, Wellehan JFX, Childress A, Norton TM, Stedman NL, Bloom DC, Belzer W, Titus VR, Wagner R, Brooks JW, Spratt J, Jacobson ER. 2008. Ranavirus infection of free-ranging and captive box turtles and tortoises in the United States. Journal of Wildlife Diseases 44: 851–863.

Junge RE, Louis EE. 2005. Biomedical evaluation of two sympatric lemur species (*Propithecus verreauxi deckeni* and *Eulemur fulvus rufus*) in Tsiombokibo Classified Forest, Madagascar. J Zoo Wildl Med. 36(4):581-9.

Kimble, SJA, Williams, RN. 2012. Temporal variance in hematologic and plasma biochemical reference intervals for free-ranging eastern box turtles (*Terrapene carolina carolina*). Journal of Wildlife Diseases 48: 799-802.

Lillywhite HN. 1987. Temperature, energetics, and physiological ecology. In: *Snakes: ecology and evolutionary biology*, Seigel RA, Collins JT, Novak S, editors. MacMillan Publishing Company, New York, New York, pp. 422-477.

Martel A, Spitzen-van der Slujis A, Blooi M, Bert W, Ducatelle R, Fisher MC, Woeltjes A, Bosman W, Chiers K, Bossuyt F, Pasmans F. 2013. *Batrachochytrium salamandrivorans* sp. nov. causes lethal chytridiomycosis in amphibians. PNAS 110: 15325-15329.

Mazet JK, Gardner IA, Jessup DA, Lowenstine LJ, and Boyce WM. 2000. Evaluation of changes in hematologic and clinical biochemical values after exposure to petroleum products in mink (*Mustela vison*) as a model for assessment of sea otters (*Enhydra lutris*). American Journal of Veterinary Research 61: 1197–1203.

Metcalf, EL, Metcalf AL. 1979. Mortality in hibernating ornate box turtles, *Terrapene ornata*. Herpetologia 35: 93-96.

Navarro-Gonzalez N, Ugarte-Ruiz M, Porrero MC, Zamora L, Mentaberre G, Serrano E, Mateos A, Lavín S, Domínguez L. 2014. Campylobacter shared between free-ranging cattle and sympatric wild ungulates in a natural environment (NE Spain). Ecohealth. 11(3):333-42.

Ossiboff, RJ, Raphael BL, Ammazzalorso AD, Seimon TA, Niederriter H, Zarate B, Newton AL, McAloose D. 2015. A Mycoplasma species of Emydidae turtles in the notheastern USA. J Wildl Dis 51: doi: 10.7589/2014-04-086.

Parsons MB, Travis D, Lonsdorf EV, Lipende I, Roellig DM, Kamenya S, Zhang H, Xiao L, Gillespie TR. 2015. Epidemiology and Molecular Characterization of *Cryptosporidium* spp. in Humans, Wild Primates, and Domesticated Animals in the Greater Gombe Ecosystem, Tanzania. PLoS Negl Trop Dis. 20;9(2):e0003529. doi: 10.1371/journal.pntd.0003529.

Peterson CR, Gibson AR, Dorcas ME. 1993. Snake thermal ecology: the causes and consequences of body-temperature variation. In: *Snakes: ecology and behavior* , Seigel RA, Collins JT, editors. McGraw-Hill Inc., New York, pp. 241-314.

Rose BMW, Allender MC. 2011. Health assessment of wild eastern box turtles (*Terrapene carolina carolina*) in east Tennessee. *J Herp Med Surg* 21: 107-112.

Schloegel LM, Hero J-M, Berger L, Speare R, Mcdonald K, Daszak P. 2006. The decline of the sharp-snouted day frog (*Taudactylus acutirostris*): The first documented case of extinction by infection in a free-ranging wildlife species. *Ecohealth* 3:35—40.

Schrader G, Allender MC, Odoi A. 2010. A retrospective study of diagnosis, treatment, and outcome of eastern box turtles (Terrapene carolina carolina) presented to the University of Tennessee College of Veterinary Medicine wildlife clinic from 1995–2007. Journal of Wildlife Diseases 46: 1079–1085

Silbernagel C, Clifford DL, Bettaso J, Worth S, Foley J.. 2013. Prevalence of selected pathogens in western pond turtles and sympatric introduced red-eared sliders in California, USA. Dis Aquat Organ. 107(1):37-47.

Sleeman J. 2008. Use of wildlife rehabilitation centers as monitors of ecosystem health. In Zoo and wild animal medicine, current therapy 6, Fowler M. E., Miller R. E. (editors.). St. Louis, Missouri: Saunders Elsevier,,pp. 97–104.

Skerratt LF, Berger L, Speare R, Cashins S, McDonald KR, Phillott AD, Hines HB, Kenyon N. 2007. Spread of Chytridomycosis has caused the rapid global decline and extinction of frogs. *EcoHealth* 4:125–134.

Turvey ST, Risley CL, Barrett LA, Yujiang H, Ding W. 2012. River dolphins can act as population trend indicators in degraded freshwater systems. PLoS One. 7(5):e37902. doi: 10.1371/journal.pone.0037902.

Williams NJ, Sherlock C, Jones TR, Clough HE, Telfer SE, Begon M, French N, Hart CA, Bennett M. 2011. The prevalence of antimicrobial-resistant *Escherichia coli* in sympatric wild rodents varies by season and host. J Appl Microbiol. 110(4):962-70.

Wright KM, Skeba S. 1992. Hematology and plasma chemistries of captive prehensile-tailed skinks (*Corucia zebrata*). Journal of Zoo and Wildlife Medicine 23: 429–432.